GOALS 2002, May 18, 20002
(penultimate)

Research conducted at MLBS in 2002 will address the effect of T on the behavior and physiology of females (the female project). We will have a relatively small crew in the field, and not all people will be there at the same time, e.g., Eric, and Wendy Wolf will be here early and Ethan and Ian will be here later. Dawn, Katie, and Jackie will provide the season-long continuity.

In the past (1989-2000), we saturated the study area with T- and C-males, observed behavior and physiology, and measured relative reproductive success and survival of males of both types. The task required that we census twice a year, map territories, find nests, bleed/band/weigh nestlings, and then remove implants at the end of summer and mark the years' new juveniles. Numerous sub-projects allowed us to measure the effects of the implants on behavior and physiology.

Beginning in 2001, we turned to implanting females and measuring the effect of T on their behavior, physiology, and fitness. The rationale is to determine the extent to which males and females resemble one another in the ways they re affected by elevated T. If T does not affect females, then, we argue, selection on T in males need not lead to a correlated response in females. If females are affected, and there is a genetic basis to the level of effect, then a correlated response to selection would be expected.

The goal is to understand the mechanisms underlying the extent to which the sexes to resemble one another, in real time and in evolutionary time.

The specific goals for 2002 are to obtain more data on the effect of T on female nest building, egg laying, incubation, caring for young, and defense against predators. Last summer there was no effect of T on incubation or nest defense, but the samples were small and we did not get to look at nestling feeding behavior. So we hope to confirm the lack of effect on incubation (and possibly nest defense) by upping the sample size. We also want to measure effect of T other forms of female behavior, e.g., aggression/song/feeding of nestlings. Finally we will look at effects of T in females on other hormones (DHEA, cort, cort binding globulin, estradiol, etc.). T elevates cort in captive females and males, but we need to confirm that in the field and look at cort binding globulin.

Listed here are goals for the summer 2002. They are in chronological order in terms of the progression of reproductive efforts. We still need to set priorities.

1. **Implant females** by catching them at random at traditional net and trap sites or by catching them off the nest. Measure and mark any males caught in the process. Bleed all adults at capture for DNA (see Process 2002)(Everybody).

2. **Map study area; try to obtain an accurate reading of all nest locations (using GPS if**

possible, as well as readings for all trap and net locations)(Katie?).

3. **T and female phenotype:** determine whether testosterone interferes with **nest- building or egg laying** simply by following females during this stage and comparing treatments for measures like time to complete nest, time between completion of nest and appearance of first egg, gaps between eggs, failure to lay, etc. We anticipate that some females may be highly affected and perhaps not lay at all, while others may be affected to varying degrees (no special protocol, usual techniques, see Nest 2002)(Eric then Ian and crew).

4. **T and female phenotype - egg steroids**: determine whether T-females produce eggs with higher concentrations of T or of different size than eggs of C-females (mark eggs during laying, collect one per clutch - 3rd egg or largest egg if laying order not known)(most nests will fail, so may still be able to observe parental behavior at full broods)(essential to know when female implanted in relation to when eggs collected)(this is not a hypothesis-driven question, but useful for interpretation and should make a short note)(see Nest 2002 for protocol)(Eric measures eggs, then Ian, crew for finding and marking).

5. **T and female phenotype:** assess effect of T on **response to a challenge** from an intruding male (or female?) conspecific during incubation (= nest defense?) and **assess effectiveness of implants** by bleeding females. Females are very aggressive towards male intruders near the nest, so half way through incubation period, we could present a male lure and use time to capture as a measure of aggressiveness, then bleed females for T. We have to determine that implants elevate T or all could be lost, so this seems like a good idea, but it could interfere with predator presentations or incubation watches, and there could be some habituation from earlier efforts to catch females off nest for implant (see protocol Response to Intruder.2002)(Wendy/Ian?)

6. **T and female phenotype: allocation to parental effort** during incubation, when rearing nestlings, in response to predator (repeat of last year's studies, to increase sample size and firm up conclusions). Also assess whether males compensate for any change in female parental effort. This could conflict with need to bleed females and assess aggression. Do we think it is possible to do two presentations during the incubation stage? Which should take priority? (Need protocol from Ethan/Dawn for incubation; need to produce protocol for nest watches?)(Dawn/Ethan)

7. **T and offspring growth and survival, also rates of EPFs and sex ratio**: effect of T on female sexual behavior/attractiveness and effectiveness of parental behavior. Determine whether T affects fitness, including EPF frequency and sex ratio of offspring (usual techniques, measuring number and quality of offspring, bleeding males, females, newly hatched young for sex ratio, and day 6 young for DNA to assess relatedness)(see nest 2002 and eggs&sex.2002)(Eric then Ian and crew).

8. **T and female phenotype - cort and corticosteroid binding globulin (CBG)** in free-living T- and C-females: collect plasma at nest leaving in usual way except make every effort to capture birds naturally and without disturbance. Assess whether T elevates cort

in females in the wild (it does in captives) and also CBG. Do this only if have bled for hormones prior to nest leaving. Need to coordinate this bleed with efforts to bleed for T during incubation)(see Nest 2002, plus Cort&CBG.2002)(Eric then Ian and crew).

9. **In addition? Add to Bloomington colony**: in late summer transport newly caught juncos to Bloomington (adults or juveniles) to help maintain the colony of juncos there.

10. **In addition? assess importance of early condition** to later fitness by obtaining plasma hormone samples from juveniles to see whether cort or T predicts which ones return. A possible good late summer project for an interested person.

For 2002-2003 (work out techniques in Bloomington this summer)

- **Heritability, repeatability on captives (or in the field):** assess whether to expect a response to selection on T in males or females by attempting to measure heritability. Transport siblings to Bloomington, compare for hormone levels, behavior, and other hormone-mediated traits, e.g., GnRH challenge. Also attempt to measure in the field using Ritland marker techniques.

- **Response of T to GnRH challenge** (measure of endogenous variation in response of T to stimulus).

- **Response of LH to T challenge** (measure of endogenous variation in response of HPG axis to T).

Remaining projects carried over from earlier years to be conducted during some other year.

- Possibly compare the response (hyperactivity) of recently captured parental males and females to tapes of begging calls.

- Measure begging response of hand-reared young to simulated treatment-specific feeding schedules to see how nestlings "learn to beg." See if this would fit with already collected data comparing T- and C-males for the schedules on which they feed their young.

- Isolate effect of T on parental behavior from effect of T on nestling begging by allowing non-T-implanted adults to feed young hatched from eggs laid by T-treated females and T-implanted adults to feed young hatched from eggs not laid by T-treated females. That is, implant some females before egg-laying and some after and remove implants from some and not from others during the nestling stage.

- Does experience with having been mated to a T-male affect whether a female finds T-males more attractive than C-males?

- Alter tail white with extra food in the nest or some other early environmental manipulation

- Measure natural variation in flexibility in response to experimentally altered mating and parental opportunities by comparing hormone levels and behavior at the nest when there is or is not a fertile female nearby or before and after their broods have been enhanced in size.

- Work up unpublished data: Quantify comparisons of molt in males whose implants were not removed. Compare pox, condition, body mass, clo pro, fat class, etc. of T- and C-males, i.e., summarize already existing data.